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In Vivo Systems Response Profiling and Multivariate Classification of CNS Active Compounds: A Structured Tool for CNS Drug Discovery

Susanna Waters,*[,†,‡] Peder Svensson,[‡] Johan Kullingsjö,[‡] Henrik Pontén,[†] Theresa Andreasson,[§] Ylva Sunesson,[§] Elisabeth Ljung,[‡] Clas Sonesson,[‡] and Nicholas Waters[‡]

[†]Department of Pharmacology, Gothenburg University, SE-405 30 Gothenburg, Sweden

[‡]Integrative Research Laboratories Sweden AB, Gothenburg SE-413 46, Sweden

[§]AstraZeneca R&D, SE-431 83 Mölndal, Sweden

Supporting Information

ABSTRACT: This paper describes the application of in vivo systems response profiling in CNS drug discovery by a process referred to as the Integrative Screening Process. The biological response profile, treated as an array, is used as major outcome for selection of candidate drugs. Dose−response data, including ex vivo brain monoaminergic biomarkers and behavioral descriptors, are systematically collected and analyzed by principal component analysis (PCA) and partial least-squares (PLS) regression, yielding multivariate characterization across compounds. The approach is exemplified by assessing a new class of CNS active compounds, the dopidines, compared to other monoamine modulating compounds including antipsychotics, antidepressants, and procognitive agents. Dopidines display a distinct phenotypic profile which has prompted extensive further preclinical and clinical investigations. In summary, in vivo profiles of CNS compounds are mapped, based on dose response studies in the rat. Applying a systematic and standardized work-flow, a database of in vivo systems response profiles is compiled, enabling comparisons and classification. This creates a framework for translational mapping, a crucial component in CNS drug discovery.

KEYWORDS: *Neurochemistry, monoamines, rodent behavior, drug discovery, systems pharmacology, phenotypic screening, multivariate data analysis, dopidines, antipsychotics, response profiles, biomarkers, translational modeling*

INTRODUCTION

Besides the therapeutic class, CNS active compounds are typically categorized either based on receptor affinities/interactions at the presumed target protein, e.g., "SSRIs" (selective serotonin reuptake inhibitors), or based on the chemical structure class, e.g., "tricyclic antidepressants". Furthermore, the target centered view on CNS active drug actions dominates how drug discovery processes are implemented in today's drug discovery organizations. These processes are to a large extent built upon high throughput in vitro screening at the molecular level as the key element.[1] It is assumed that one has identified relevant molecular target(s) for intervention; and the physicochemical interaction between the target molecule and the drug molecule as defined by certain in vitro binding assays, is the prime aspect of interest. Thus, the ideal properties of a new chemical entity are defined from the start, i.e., a substance with a high and selective affinity to, and a prespecified activity at, the selected target.

However, due to limitations in the in vitro characterization, and to the complexity arising from intracellular and circuitry level downstream effects, redundancy, and adaptations occurring in vivo but not in vitro, it can be argued that there is no straightforward one-to-one relationship between in vitro and in vivo effects, and hence, in general limited knowledge on how activities on targets measured in vitro relates to effects in disease states in vivo.[2,3] A recent evaluation by Swinney and Anthony of the effectiveness of drug discovery strategies suggests a higher success rate for phenotypic vs conventional screening with respect to new drug approvals.[4] Their detailed analysis showed that the overall result was actually even more pronounced for the CNS therapeutic area. It is worth noting that their analysis is based on the total outcome from both strategies and no compensation was made for the fact that only a minute fraction of the total resources was spent on phenotypic screening.

Furthermore, mathematical modeling of biological regulatory networks suggests these are inherently resistant to perturbations of single targets/nodes,[5] which could be a major factor underlying the lack of success in finding novel, highly selective, single target treatments for CNS disorders. Polypharmacology strategies have been suggested to overcome this.[6,7] Indeed, the average number of targets among approved drugs is 6−8,[8,9] which represents a "promiscuity enrichment" of compounds reaching the market, as compared to the overall pool of

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bioactive compounds from medicinal chemistry sources, with an average of 1.9 targets/molecule.[9] Such an outcome would not be expected given the presumptions of superior efficacy and safety with single target compounds, but rather supports a multitarget approach, or, in some cases, may reflect that the drug molecule properties providing the beneficial therapeutic effects in fact arise from "off-target" activities. While it can be argued that no fundamentally new treatments have yet been developed, based on a "multitarget" approach, it is clear that combination of drugs with different modes of action, such as the use of antipsychotics combined with antidepressants,[10] very often offers important improvements of overall efficacy. Another example of successful polypharmacology in the CNS area is the novel analgesic compound tapentadol, combining two distinct action mechanisms resulting in adequate efficacy with reduced side effect compared to "pure" opioids.[11]

In view of the theoretical as well as factual shortcomings of strictly target-based drug discovery strategies, and the fact that the actual mechanisms underlying CNS disorders are to a large extent not known, it makes sense to explore the utility of different screening approaches in drug discovery, with focus on the assessment of drug effects at the system level, i.e., in the integrated physiological systems. Such assessment has the obvious advantage that it will reveal any disease related drug effect regardless if the underlying mechanism is known or not, providing that the measured response profiles are sufficiently sensitive to the effect. The structure and functional organization of physiological systems, i.e., the actual substrate for in vivo effects, are to a large extent conserved across species. For instance, the organization of the basal ganglia is conserved throughout vertebrate phylogeny,[12] as is the monoamine system as a whole.[13,14] A useful in vivo screening process should capture physiologically relevant effects, and enable multiple head-to-head comparisons of different compounds and translation to humans. In the following, we describe the practical application of such an in vivo based approach, with potential to facilitate translational modeling and improve prediction of clinical properties of novel candidate drugs.

This paper presents a process for the core pharmacological evaluation performed in the context of drug discovery of CNS active compounds which we refer to as the Integrative Screening Process (ISP). ISP is tailored to optimize the outcome of the drug discovery process by parallel assessment of key biological measures, as opposed to sequential filtering as applied in conventional drug screening programmes.[3] ISP applies CNS systems pharmacology[15] in the sense that the in vivo biological response profile, i.e., the dose dependent effects on a range of biomarkers, is the major driver in candidate selection and structure−activity relationship (SAR). Importantly, the in vivo biomarkers are treated as an array, rather than focusing on single biomarkers, which enables simultaneous assessment of many variables (in this case 228−248 measured variables), captures information residing in correlation patterns, improves signal-to-noise, enables quality control and outlier detection, and provides sensitivity and robustness.[16,17] This methodology gives the advantage of taking into account not only isolated receptor level effects of a compound, but also interactions that emerge on multiple levels in the biological system, including, e.g., downstream effects along neuronal pathways, and synergies with respect to pharmacological effects, arising from interactions with multiple targets. By systematically assessing and comparing the response profiles of different compounds, in a standardized fashion, this can be related to clinical features, i.e., therapeutic effects and adverse effects, providing a means for translational modeling and predictions.

Biomarkers include neurochemical indices related to monoamines, which are known to be key modulators of essential CNS functions including voluntary movement, feeding, affect, reward, sleep, arousal, attention, sensory processing, neuroendocrine functions, and cognition.[14,18] Monoamine systems are conserved across mammals;[13,14] and monoamines and their metabolites can be measured with high precision in different brain areas. Furthermore, descriptors of locomotor activity, which directly reflects fundamental aspects of motor function and mental state, are included, as elegantly phrased in a review by Robbins: "Behaviour is of course the main output and function of the brain. It stands to reason that any study of the brain that leaves out behavioural measurement is likely to be incomplete."[19] An application of phenotypic characterization for CNS drug discovery based only on behavioral analysis was recently published,[20] and the usefulness of multivariate behavioral assessment to study drug effects is described, e.g., by Geyer and Paulus,[21] but no attempts to combine both neurochemistry and behavior have, to our knowledge, been made before.

In the present study, data on a wide variety of known therapeutic agents including antipsychotics, antidepressants, anxiolytics, and psychostimulants as well as investigational and tool compounds, are collected, and then used to define a multidimensional compound "map" serving as a guide toward the sought after in vivo profile. Applying these principles, we have compiled an extensive database on standardized phenotypic response profiles on psychotropic compounds, which can be exploited in different areas of CNS pharmacology and drug discovery. To date, the database covers more than 350 CNS active reference compounds, including >100 compounds in clinical use in therapeutic areas such as schizophrenia, affective disorders, neurodegenerative disorders, epilepsy, and ADHD.

This method was instrumental in the discovery of dopidines, a novel class of compounds with certain modulatory effects on dopamine transmission that leads to "psychomotor stabilization", i.e., the ability to suppress motor activity in states of hyperactivity, and enhance motor activity in hypoactive states.[22−26] This class of compounds was discovered in an effort to find novel treatments for psychiatric and neurological disorders, taking into account the vital impact of central monoaminergic and specifically dopaminergic pathways in the cortico-subcortical circuitry regulating psychomotor functions, and the convergence of different pathophysiological mechanisms in, e.g., psychiatric and movement disorders toward frontal-cortical functions.[27] In short, the target preclinical profile was as follows: (1) No interference with spontaneous locomotor patterns over a wide dose range; (2) normalization of states of hypoactivity; (3) normalization of states of hyperactivity; and (4) effects primarily through the dopamine system. The objective was to find new drugs providing superior efficacy, but avoiding the troublesome side effects hampering the use of available monoamine modulating drugs, especially the motor depressant effects of antipsychotics, believed to arise from excessive dopamine blockade.[28,29] In this paper, we aim to describe in more detail the phenotypic response profiling used, and demonstrate multivariate maps, including classification of compounds assessed and tentative interpretations in terms of clinical properties as well as receptor level mechanisms underlying the in vivo profiles. Comparator compounds were

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Table 1. Overview of PLS Model on Neurochemical and Behavioural Dose Response Data on 67 Compounds[a]

component	R2X	R2X (cum)	eigenvalue	R2Y	R2Y (cum)	Q2	Q2 (cum)	significance	iterations
1	0.200	0.2	49.7	0.012	0.012	0.0116	0.012	R1	14
2	0.114	0.315	28.4	0.009	0.022	0.0084	0.020	R1	18
3	0.073	0.388	18.2	0.008	0.030	0.0057	0.025	R1	70
4	0.065	0.453	16.1	0.007	0.037	0.0050	0.030	R1	200
5	0.059	0.512	14.5	0.008	0.045	0.0065	0.037	R1	23
6	0.040	0.552	9.78	0.007	0.052	0.0050	0.041	R1	36
7	0.039	0.591	9.74	0.005	0.057	0.0024	0.044	R1	62
8	0.046	0.637	11.4	0.003	0.060	0.0005	0.044	R1	72

[a]R2X: Fraction of variance in *X* block explained by each component, and cumulative (R2Xcum). R2Y: Fraction of variance in *Y* block explained by each component, and cumulative (R2Ycum). Q2 denotes the overall cross-validated R2 for each component, and cumulative (Q2cum). All components were statistically significant as determined by cross-validation (Q2 > 0, denoted R1).

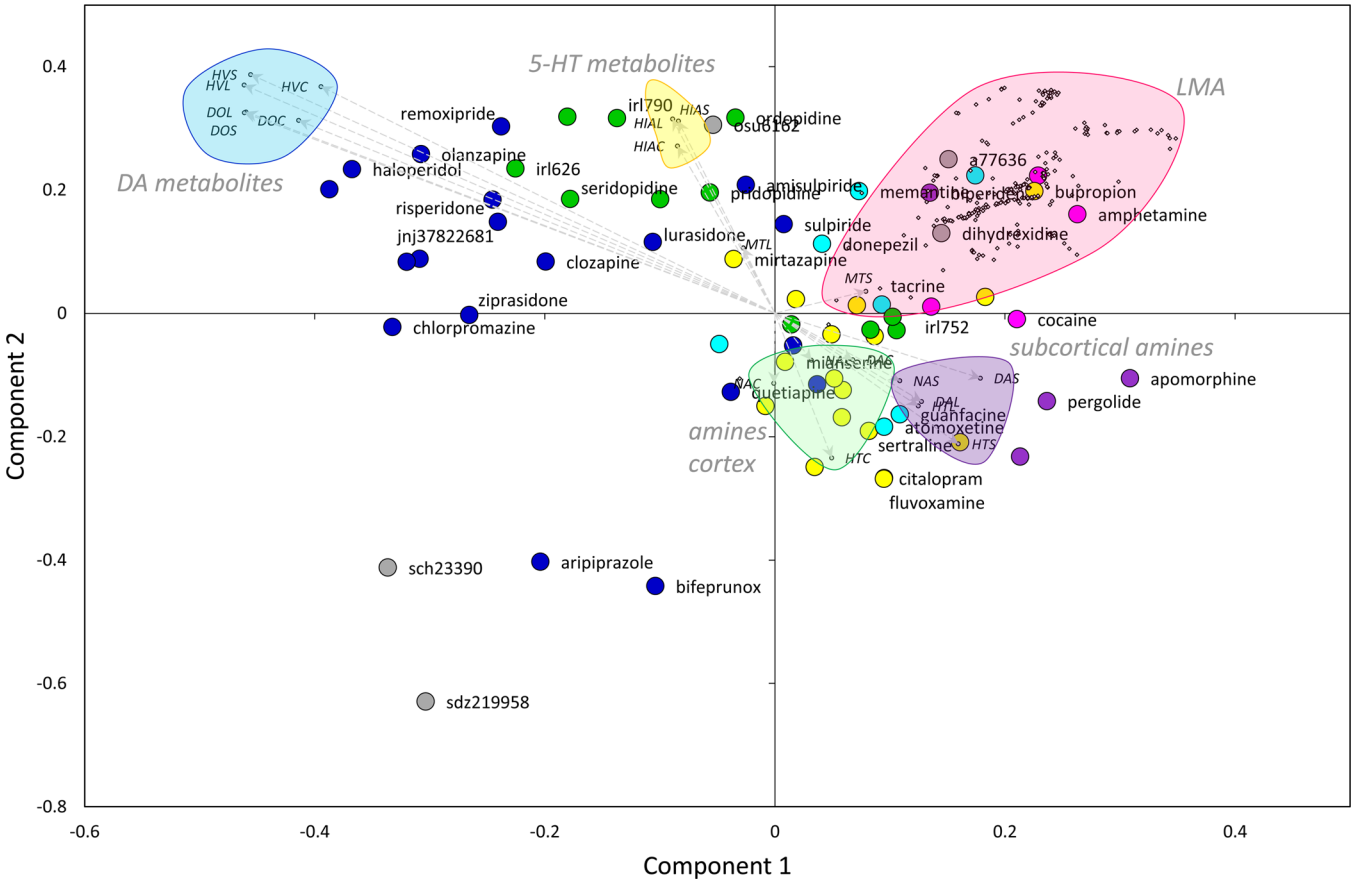

Figure 1. Variable weights (*w***c*) from PLS regression model based on dose−response data on neurochemistry and behavior for 67 compounds. Shown are dependent (*Y*) variable weights along component 1 and 2 (colored circles), superimposed on vectors representing independent (*X*) variable weights for the neurochemical variables, and dots representing the behavioral variable weights. *X*-Weights are scaled to optimize readability, applying scaling factor of 1.5 for neurochemistry variables and 5 for behavioral variables. Areas with closely related clusters of variables have been encircled and shaded to enhance readability. Briefly, the location of each *Y* variable (compound) represents the overall direction of the dose dependent effects of that particular compound on the underlying variables, i.e., compounds located close to each other have similar effects. Coloring represents compound class: Green, in-house compounds; blue, antipsychotics; yellow, antidepressants; purple, DA agonists/PD drugs; gray, DA D1 ligands; pink, abuse; turquoise, procognitive/ADHD.

selected to span a variety of pharmacological profiles and therapeutic classes, representing typical and atypical antipsychotics, antidepressants, procognitive agents, and psychostimulants. In addition to the dopidines, data on a further set of compounds discovered by the phenotypic screening principles outlined herein are included: IRL547, IRL790 (psychomotor stabilizers), IRL626, IRL678 (fast-off DA D2 antagonists),[30,31] IRL667,[32] IRL696,[33] IRL744, and IRL752 (referred to as cortical enhancers).[34]

RESULTS AND DISCUSSION

We have applied partial least-squares (PLS) regression analysis of systematically collected phenotypic in vivo response data, to obtain variable weight plots which serve as maps representing the overall pattern of effects of a wide range of psychotropic compounds. This is demonstrated by two different models: one more broad-ranging in terms of compound classes included and response variables and a second, more narrow, restricted to compounds primarily modulating dopamine transmission. The

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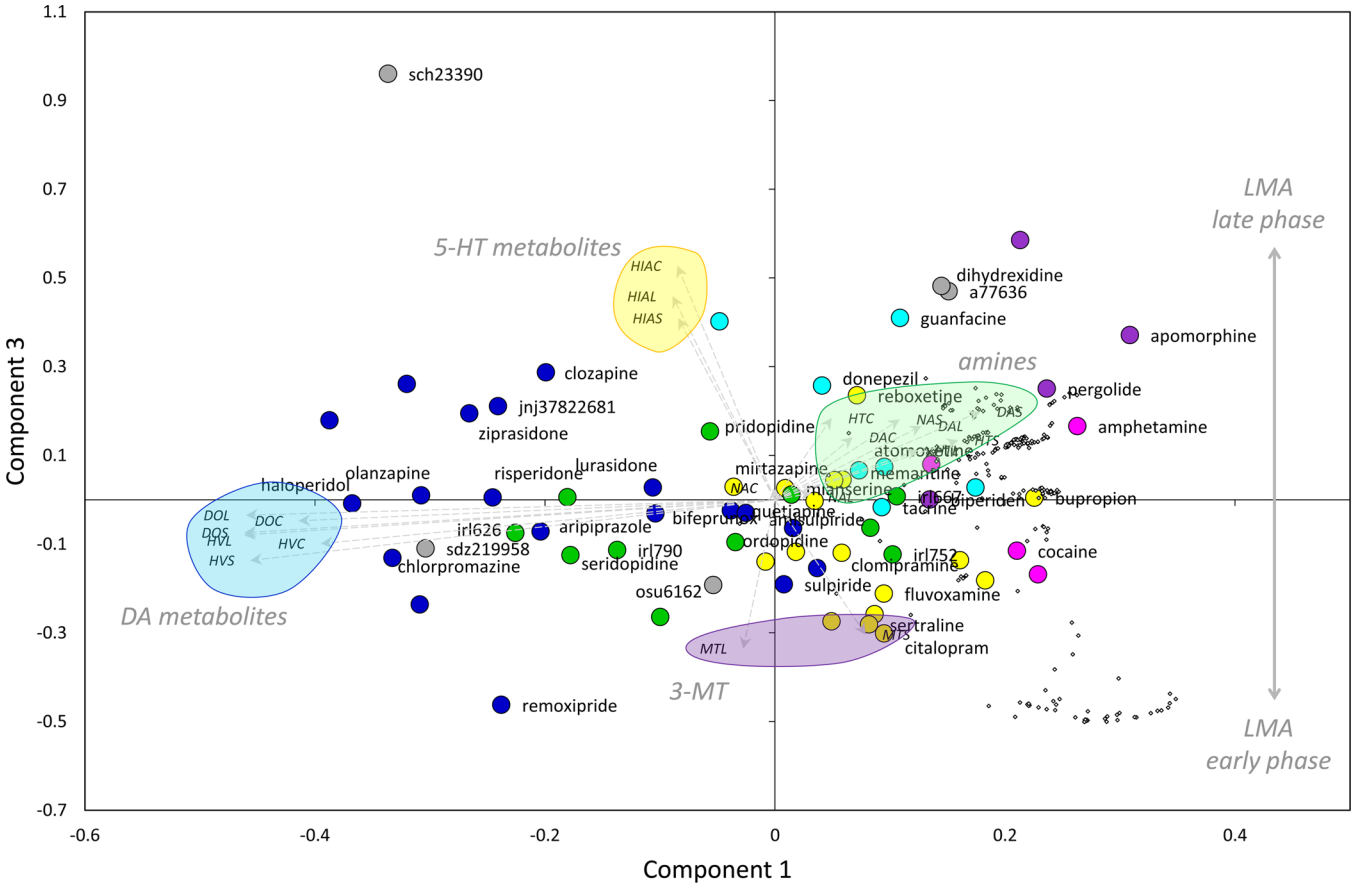

Figure 2. Component 1 vs 3 variable weights ($w*c$) from PLS regression model based on dose−response data on neurochemistry and behavior for 67 compounds. Shown are dependent (Y) variable weights (colored circles) along component 1 (horizontal) and 3 (vertical), superimposed on vectors representing independent (X) variable weights for the neurochemical variables, and dots representing the behavioral variable weights. X-Weights are scaled to optimize readability, applying scaling factor of 1.5 for neurochemistry variables and 5 for behavioral variables. Areas with closely related clusters of variables have been encircled and shaded to enhance readability. Briefly, the location of each Y variable (compound) represents the overall direction of the dose dependent effects of that particular compound on the underlying variables, i.e., compounds located close to each other have similar effects. Coloring represents compound class: Green, in-house compounds; blue, antipsychotics; yellow, antidepressants; purple, DA agonists/PD drugs; gray, DA D1 ligands; pink, abuse; turquoise, procognitive/ADHD.

analysis is focused on the properties of dopidines, a class of compound developed by this in vivo phenotyping approach, in relation to other types of dopamine modulating compounds, in particular antipsychotics.

The broad-ranging main multicompound PLS model generated was based on monoaminergic neurochemical indices and behavioral descriptors on antipsychotics, antidepressants, procognitive agents, psychostimulants, antiparkinson drugs, dopidines, and a set of compounds from a new series, referred to as cortical enhancers.[34] A significant eight component model, describing 64% of the variability in the independent variables (X) and 8% of the variability in the dependent variables (Y) was obtained (R^2X 0.64, R^2Y 0.06, Q^2 0.044; model statistics are shown in Table 1). In the typical use of PLS regression modeling of a response modeled by a set of independent variables, a low R2Y would indicate a poor fit to the model. In this case, the low degree of variability explained in Y is due to the orthogonal nature of the constructed Y-data, consisting of a matrix with one column per compound with zeros except for the rows corresponding to the specific compound (experiment) where the doses for each animal are coded (see subsection Data Analysis under Methods for more details). The purpose of the PLS models presented here is not to obtain a model for prediction but rather to describe similarities and differences between dose response data obtained for each compound. The constructed Y matrix enables a transformation from a data set of responses for individual animals to a weight map where the compounds (dose variables) are projected on top of the response (X) variables in order to summarize and display the patterns of the dose response profiles for different compound classes. The overall correlation structure of dependent vs independent variables is shown in Figures 1 and 2, showing $w*c$ weights for the first three components, i.e., weights for both X and Y variables superimposed in the same plot to visualize how effects on X variables (in vivo biological response profiles) relate to the orientation of Y variables (compound dose vectors) in the plot. Compounds are colored according to pharmacological/therapeutic class; however, to enhance readability, not all compound names in each class are written out in the graphs. Complete $w*c$ weights for all components extracted are provided in the Supporting Information Table S1. Since the Y variables in this case represent increasing doses of the test compounds analyzed, the Y variable weights c represent the direction of dose dependent effects, in relation to the effects of other compounds included in the model. Thus, each dose response profile is summarized in one c weight vector. Compounds located close to each other have similar dose dependent effects on the response profiles. On the whole, this

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Table 2. Overview of PLS Model on Behavioural Dose Response Data on 26 Compounds[a]

component	R2X	R2X (cum)	eigenvalue	R2Y	R2Y (cum)	Q2	Q2 (cum)	significance	iterations
1	0.48	0.48	109	0.031	0.031	0.029	0.029	R1	9
2	0.14	0.61	30.9	0.019	0.050	0.016	0.0446	R1	15
3	0.09	0.70	20.8	0.013	0.063	0.009	0.0533	R1	23
4	0.06	0.76	13.3	0.012	0.076	0.007	0.0596	R1	52

[a]R2X: Fraction of variance in *X* block explained by each component, and cumulative (R2Xcum). R2Y: Fraction of variance in *Y* block explained by each component, and cumulative (R2Ycum). Q2 denotes the overall cross-validated R2 for each component, and cumulative (Q2cum). All components were statistically significant as determined by cross-validation (Q2 > 0, denoted R1).

type of analysis provides a visualization of overall effects, similarities and differences among the dose dependent effects of the compounds analyzed. As an example, most antipsychotics produces dose dependent increases of dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), combined with reduced locomotor activity. This pattern of effects shows up as a clustering of these compounds (blue dots) in the upper left quadrant of Figure 1. The underlying biological response variables governing this are oriented in the corresponding direction: The DOPAC and HVA weight vectors are in the same quadrant, and the overall "cloud" of locomotor activity variables is located in the opposite direction along component one, i.e., to the right in the graph, reflecting that these compounds share a dose dependent reduction of these measures. Accordingly, the partial agonists analyzed, aripiprazole and bifeprunox, which lack the increase in dopamine metabolites, but share the inhibitory effect on locomotor activity, appear in the lower left quadrant in this graph (Figure 1, located in an orthogonal direction vs DOPAC/HVA, i.e., no dose dependent effects on these measures, but diametrically opposed to locomotor activity variables due to dose dependent behavioral inhibition). Overall, the main model reveals an overall clustering of compounds, which agrees well with the clinical properties of the compounds (manifested here by the overall therapeutic class assignments) (Figures 1 and 2). Of note, the antipsychotic quetiapine is located very close to the antidepressants (Figure 1), and thus based on this could be expected to show antidepressant like effects in vivo, which has in fact been corroborated in clinical studies, resulting in its use for major depressive disorder.[35] (The other two blue-coded compounds close to quetiapine in Figure 2 are experimental compounds, LY354740 and norclozapine both previously considered as potential antipsychotics but now discontinued for that indication.) The positions (variable weights) are governed by the direction of effects on the response variables. In particular, behavioral effects influences the position along the horizontal axis (component 1) in this model, with inhibitory compounds to the left and stimulant compounds to the right. Increases in dopamine metabolites (DOPAC, HVA) pull the neuroleptic compounds toward the upper left corner (Figure 1), while 5-hydrocyindoleacetic acid (5-HIAA) increases pull some compounds (dopidines, other IRL compounds, amisulpride) upward along component 2 (Figure 1), and 5-HIAA decreases pull antidepressants downward (components 2 and 3) (Figures 1 and 2, respectively).

As a general characteristic, dopamine D2 antagonists/partial agonists (blue) and DA D1 antagonists (gray) are located to the left, while dopamine agonists (D2/mixed, purple; D1, gray) and stimulants (pink) lie to the right (Figure 1). There is also a vertical pattern, with D1 agonists shifted upward and antagonists shifted downward (Figure 1). Antidepressants occupy an area intersected between antipsychotics and stimulants, shifted somewhat downward, while the ADHD/cognitive enhancing compounds (turquoise) are located above these. A number of compounds developed in-house (green), including the dopidines, are located either close to the cluster of ADHD/cognitive enhancers (e.g., IRL752) or just to the right of the main "antipsychotics" cluster (e.g., pridopidine, ordopidine, IRL790). Looking at the third component (Figure 2), it appears that this contributes to the separation of antidepressants vs ADHD/cognitive enhancers, related to, e.g., differential effects on 5-HIAA (positive weights on component 3), 3-MT (negative weights), and some of the behavioral measures (negative weights for activity late in the recording session, positive weights for activity in the early phase, reflecting differential effects over time among compounds separated along component 3).

Features Relating to DA D1/D2 Receptor Activity. In the first projection shown (Figure 1), which represents the two first components, accounting for the largest part of the variance modeled, a pattern strongly relating to dopamine D1 and D2 receptor effects appears to be present. There is a left-upward axis oriented along D2 antagonist/agonist-like effects, and an orthogonal, left-downward axis oriented along D1 antagonist/agonist-like effects (Figure 1). This pattern is of course related to the inclusion of several antipsychotics with strong D2 antagonist effects such as dose dependent DOPAC and HVA increases, which have a great impact on the model, however the presence of an orthogonal direction potentially related to D1 receptor agonist/antagonist-like effects affecting the orientation of different compound classes included, suggests not only independent net system level effects of selective D1 vs D2 ligands, but also that unrelated, nondopaminergic compounds can display similar effects in vivo. Thus, several cognitive enhancers, including memantine, a NMDA antagonist, and donepezil, an acetylcholinesterase inhibitor, are located in the DA D1 agonist direction in this projection (Figure 1), and are also located close to the D1 agonists in the component 1 vs component 3 projection (Figure 2). Looking at the underlying response variables, a tendency toward behavioral activation, combined with altered tissue amine levels are noted. These cognitive enhancers have been shown to enhance dopamine transmission, e.g., in the frontal cortex,[36] which could explain their similarities with DA D1 agonists in a global in vivo assay, which captures net system level effects rather than specific receptor protein interactions. A set of in-house compounds, exemplified by compound IRL752, the main effects of which are to increase extracellular NA and DA, as well as *Arc* mRNA in the frontal cortex,[34] also cluster among the cognitive enhancers (Figure 1). Furthermore, the dopidines are shifted toward this area, compared to the cluster of classical DA D2 antagonists, which would be consistent with similar effects, i.e.,

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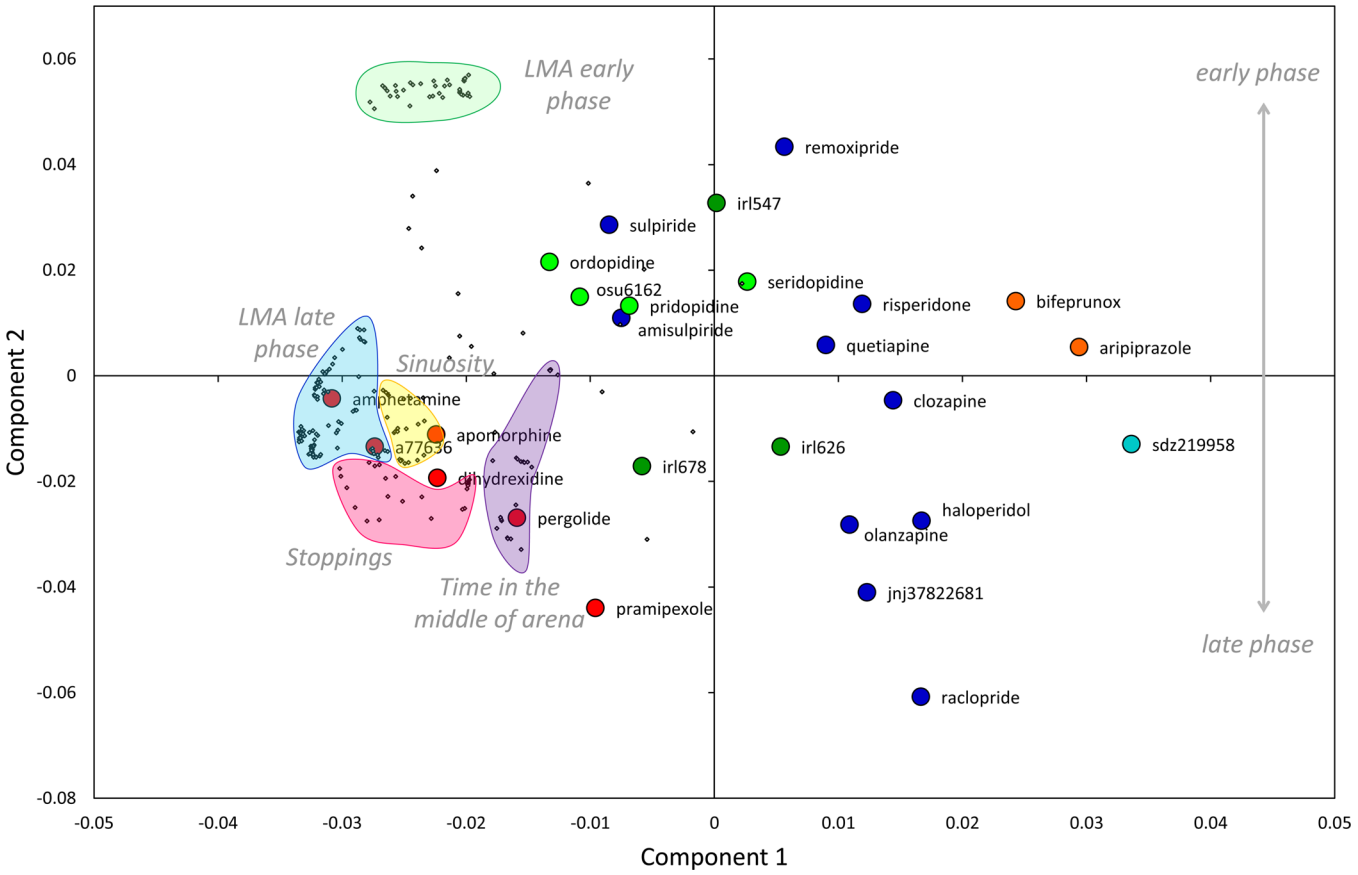

Figure 3. Variable weights ($w*c$) from PLS regression model based on dose−response data on behavior for 26 compounds. Shown are dependent (Y) variable weights along component 1 and 2 (colored circles), superimposed on dots representing variable weights for the independent (X) variables (308 behavioral descriptors). X-weights are scaled to optimize readability, applying scaling factor of 0.4. Areas with closely related clusters of variables have been encircled and shaded to enhance readability. Briefly, the location of each Y variable (compound) represents the overall direction of the dose dependent effects of that particular compound on the underlying variables, i.e., compounds located close to each other have similar effects. Coloring represents compound class: Bright green, dopidines; dark green, other in-house compounds with fast-off DA D2 receptor dissociation kinetics; blue, antipsychotics; red, dopamine agonists; turquoise, D1 antagonists; orange, partial DA agonists. LMA: Locomotor activity.

in the DA D1 agonist like direction, superimposed on the DA D2 antagonist neurochemical profile of the dopidines.

A second more focused PLS model was generated focusing on compounds more specifically modulating dopamine transmission as the primary effect, and restricting the analysis to behavioral measures, to obtain a more detailed analysis of, in particular, the differences between the behavioral effects of different types of antipsychotics and dopidines. This model was based on data on 26 compounds, including antipsychotics, dopidines, dopamine D1 and D2 agonists and antagonists, and dopaminergic stimulants. PLS regression yielded a four component model, capturing 76% of the variability in the X block, explaining 8% of the variability in Y (Table 2). A $w*c$ weight plot, showing X and Y variable weights superimposed, for the first two components, is shown in Figure 3. $w*c$ weights for all components extracted are provided in Supporting Information Table S2.

Component 1 (horizontal axis) essentially represents overall impact on the level of locomotor activity, with compounds increasing activity to the left and compounds decreasing activity to the right. Variables capturing time spent in the central part of the arena are also to a large extent represented by the first component, indicating that this correlates to overall behavioral activation among the compounds tested. The second component (vertical axis) is related to the time-course of the locomotor effects, with variables capturing early phase activation in the upper half. Furthermore, some behavioral pattern variables such as stoppings and time spent in the center of the arena have significant, negative, weights along component 2, which contributes to the separation of compounds depending on their effects on these measures. Here, the antipsychotics (DA D2 antagonists, blue; partial agonist, orange) are located in the right half of the graph, due to general inhibitory effects on locomotor activity among these compounds; in accordance with the outcome of the previous PLS model. One notable exception is the benzamides; sulpiride and amisulpride, located close to the dopidines (bright green), known to lack inhibitory effects on locomotion in the normal state.[22,23] There is also a set of in-house compounds not classified as dopidines (dark green); acting as DA D2 antagonists with fast dissociation kinetics[31,30]. Of note, unlike the other D2 antagonists assessed, including the compound JNJ-37822681, which is also reported to be a fast dissociating D2 antagonist,[37] these compounds do not inhibit locomotor activity. The most profound locomotor inhibitory effects in this data set are observed for the partial DA agonists (orange), aripiprazole and bifeprunox, along with the DA D1 antagonist tested, SDZ219-958 (turquoise), displaying very similar behavioral effect profiles (located together in the far right end along component 1). The high affinity D2 antagonist

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antipsychotics (blue), except the benzamides, are located in the lower right quadrant, suggesting relatively more inhibitory effects during the early recording phase. Clozapine and quetiapine, antipsychotic compounds with lower D2 affinity, display less marked effects (smaller weights along component 1) compared to the partial agonists, and less impact on early phase locomotor activity compared to the group of high affinity D2 antagonist; however, a similar profile was also observed with risperidone, an atypical antipsychotic with high affinity at DA D2 receptors.

Regarding the full agonists and stimulants (red), D-amphetamine displayed the most marked stimulatory effect on behavior (Figure 3, large negative *c* weight along component 1). The D1 and mixed D1/D2 agonists are clustered in the direction of sinuosity, stoppings, and time in the center of the arena, while this is less evident for the D2 selective agonists pergolide and pramipexole. Furthermore, the D2 selective agonists displayed less overall behavioral activation compared to the other stimulant compounds. Finally, the dopidines (bright green) and a predecessor compound, (−)-OSU6162,[38] are clustered in an area rather close to the origin, oriented somewhat to the left in the graph, essentially in the direction of early phase activity, indicating no behavioral inhibition, but possibly some slight activation, however with a pattern distinct from the stimulants which are located in an almost orthogonal direction.

This second more focused behavioral model provides a demonstration of "zooming" in the multivariate map, by focusing on one domain of biological descriptors, and restricting the set of test compounds (Figure 3). In this case we used primarily dopamine modulating compounds, selected to cover D1 and D2 selective ligands as well as dopidines and antipsychotics representing both typical and atypical ones. Among the direct and indirect agonists (red), all displayed clear behavioral activation, with qualitative features including increased sinuosity, stoppings, and activity in the center of the arena distinguishing D-amphetamine, D1 selective agonists and the mixed D1/D2 agonist apomorphine from D2 selective compounds (pramipexole, pergolide). Not surprisingly, D-amphetamine induces the most impressive behavioral increase, reflected by the large negative weights along component 1 (left-most position on the horizontal axis). Conversely, the dopamine antagonists in most cases decreased locomotor activity, albeit with qualitative and quantitative differences among the compounds tested. High affinity D2 antagonists such as haloperidol, raclopride and JNJ-37822681, as well as olanzapine, are clustered in the lower right corner reflecting overall inhibitory effects especially in the early phase of the recording session. The D1 selective antagonist SCH23390 (turquoise), as well as the partial agonists aripiprazole and bifeprunox, exert a qualitatively different behavioral inhibition, mainly affecting late phases. An intermediate profile was observed with clozapine, quetiapine, and risperidone, displaying less profound inhibition, albeit qualitative more similar to the partial agonists than to the high affinity D2 antagonists, as revealed by the orientation of these compounds essentially along component 1. Compared to the main model, this analysis enables a clearer distinction between the specific behavioral profiles of different compounds, for instance it demonstrates that the partial agonists differ from other antipsychotics not only due their relative lack of neurochemical effects, but also in terms of behavioral qualities. It also helps distinguishing dopidines and other dopamine modulatory compounds which have DA D2 antagonist properties in vitro and with respect to neurochemistry, but are clearly different from both classical D2 antagonists and partial agonists with respect to their behavioral effects, which rather display some resemblance to the dopamine agonists. It was recently shown that dopidines, as opposed to classical D2 antagonists as well as partial agonists and atypical antipsychotics, increase Arc mRNA, a marker of synaptic activation,[39] in the frontal cortex; an effect which may be of relevance to the specific, state-dependent, behavioral effects of the dopidines.[22]

Antipsychotics. The antipsychotic compounds occupy a wide area in the main model (Figures 1 and 2), reflecting considerable differences with respect to behavioral activity, which is inhibited by several but not all of these compounds, and neurochemical indices, especially dopamine metabolites (DOPAC, HVA) and serotonin metabolites (5-HIAA). Some degree of correspondence between major in vitro binding properties and in vivo response profiles can be discerned. As discussed above, D2 antagonists tend to cluster in the upper right corner (main model, Figure 1). Aripiprazole and bifeprunox, described as partial D2 agonists, have a distinct position toward the lower right corner (Figure 1), reflecting a combination of behavioral inhibition and absence of increases in DOPAC and HVA displayed by these compounds. Furthermore, this location represents a similarity with DA D1 receptor antagonists, also evident by their position in the opposite direction vs the D1 agonists. Possibly, partial agonism at D2 receptors creates a net effect resembling that of DA D1 antagonists, although neither aripiprazole nor bifeprunox acts as D1 antagonists in vitro. To ensure that this phenomenon did not arise due to excessive doses of the partial agonists, leading to unspecific behavioral inhibition, we checked the behavioral effects in the lowest doses tested, also in replicate experiments, and found that the behavioral inhibition, e.g., by aripiprazole appears in the same dose range as has been reported to increase DA efflux.[40] Tentatively, one explanation of the "D1-antagonist-mimicking" effect could be functional selectivity vs DA receptor signaling pathways.[41] The main point in this context is that the model picks up this effect very efficiently applying the multivariate profiling approach.

Impact of DA D2 Receptor Dissociation Kinetics. Apart from partial agonism, fast receptor dissociation kinetics has been put forward as a key property that distinguishes atypical from typical D2 receptor antagonists especially with respect to EPS liability which has been attributed to excessive D2 receptor blockade.[42] Among the compounds tested here, the antipsychotics quetiapine, clozapine, and JNJ-37822681, as well as the dopidines, and the in-house compounds IRL626 and IRL678 are reported to display fast-off D2 antagonism.[30,37,42,43] However, these "fast-dissociating" D2 antagonists span most of the antipsychotics area. In Figure 1, JNJ-37822681 (k_{off} = 0.11 s^{-1})[37] is located among the left-most of the antipsychotic compounds, e.g, haloperidol, a high-affinity antagonist not classified as fast dissociating (k_{off} = 0.01 s^{-1}),[37] while quetiapine sits close to the origin, i.e., in the extreme right border of the antipsychotics, and clozapine (k_{off} = 0.05 s^{-1})[37] is located in between. The dopidines and IRL denominated compounds displaying fast-off D2 receptor dissociation kinetics are located upward, relative to these compounds (k_{off} not calculated; fast dissociation judged by fast and complete recovery of DA responses after wash-out[30]). In the model based on behavior only (Figure 3), again, clozapine takes an intermediate position between the more inhibitory compound JNJ-37822681, and

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quetiapine, whereas the dopidines and IRL D2 modulators have a location above these, close to the benzamides sulpiride and amisulpride. Hence, fast-off dissociation as such is not associated with any specific overall profile (Figures 1 and 2) or behavioral profile (Figure 3). Some discrepancies with respect to the in vitro binding have been pointed out for, e.g., JNJ-37822681, which was reported to display lack of reversibility in terms of functional dopamine responses[43] measured on whole cells, as opposed to the rapid dissociation rate reported using purified membranes.[37] The functional recovery study concluded that there is no clear association between rapid recovery rates and atypicality. However, it is worth noting that the compounds displaying complete recovery of functional dopamine responses, i.e., sulpiride, remoxipride, pridopidine, and (−)-OSU6162, all are located in the "non-inhibitory" cluster in the upper, left area of Figure 3, suggesting some correspondence between the in vitro finding and behavioral effects in vivo. On the other hand, risperidone, a high affinity D2 antagonist, with k_{off} = 0.004 s^{-1},[37] and lacking functional recovery of dopamine responses,[43] displays limited behavioral inhibition, as reflected by its position in the central region of Figure 3, and is clearly offset from the other high affinity D2 antagonists with strong behavioral inhibition such as haloperidol or JNJ-37822681.

Clinical Profile of Antipsychotics. Considering the clinical profile of the different antipsychotics assessed, we have used the results from recent meta-analyses[44,45] comparing currently used antipsychotics with respect to overall efficacy in the treatment of schizophrenia, as well as side effect liability to guide our interpretations of the present findings. These analyses concluded that clozapine and amisulpride stand out as the most efficacious antipsychotic drugs, followed by olanzapine. This corresponds to a broad, intermediate region in the main model (Figure 1), overlapping the dopidines, but not extending to the left-most region where the strong DA D2 antagonist compounds (haloperidol, raclopride) are found. On the other hand, the meta-analyses suggest chlorpromazine, lurasidone, and ziprazidone to be among the least efficacious compounds, and these are located further down in the main model, chlorpromazine and ziprazidone to the left due to behavioral inhibition, lurasidone more toward the middle (Figure 1). In the behavioral model, the most effective compounds, clozapine and amisulpride, appear in an intermediate region, covering compounds with behavioral effects ranging from very subtle, stimulant to locomotor inhibition, albeit not overlapping the most inhibitory compounds (Figure 3). On the whole, antipsychotics display a highly variable degree of behavioral inhibition, which does not correlate with overall antipsychotic efficacy, although it is noteworthy that the most efficacious compounds are found among the ones with limited behavioral inhibition.

EPS liability has been attributed to high-affinity dopamine D2 antagonism, and further, hypothesized to be influenced by several factors such as serotonergic components of antipsychotic drug effects,[46] as well as the receptor dissociation kinetics and degree of intrinsic activity, e.g., at DA D2 receptors.[29] However, second and third generation antipsychotic drugs developed based on these ideas are still considered to induce EPS.[47] The meta-analyses considered herein as a guide on overall clinical properties indicates clozapine to be relatively free of EPS. Older compounds such as haloperidol and risperidone, as well as chlorpromazine, have the most clear-cut EPS liability, whereas newer compounds appear to carry an intermediate risk.

Looking at the main model (Figure 1), the compounds with high EPS liability tend to be located to the left, driven by more pronounced increases in dopamine metabolites, as compared to the low liability compounds. The behavioral effect profile, at least as captured in the two first components of our behavioral model, does not appear to discriminate between high and low EPS liability compounds (Figure 3). However, the ranking of different compounds with respect to EPS liability is not clear-cut, and some studies indicate no major differences, e.g., between first and second generation antipsychotics.[47,48] Furthermore, EPS is also observed in drug-naïve schizophrenic subjects.[49]

As to the sedative properties of current antipsychotics, the situation is less equivocal. Most antipsychotics, including clozapine, olanzapine, and quetiapine as well as typical antipsychotics are clearly prone to induce sedation, with the notable exception of amisulpride and aripiprazole, which appear to carry a very low risk for this side effect.[44] Second generation antipsychotics, in particular quetiapine, are frequently used to treat insomnia.[50] In the overall model, the compounds typically associated with sedation, including clozapine, chlorpromazine, and quetiapine, are found in the intermediate region, separated from both amisulpride and aripiprazole (Figure 1). Concerning amisulpride, the lack of behavioral inhibitory properties appears to be a key feature translating to lack of sedation in humans. Accordingly, the other benzamides with a similar profile, sulpiride and remoxipride, are also considered to be relatively nonsedative.[51,52]

In summary, it appears that the most efficacious antipsychotic compounds can be picked from a wide, intermediate area (Figure 1), and, importantly, that this does not overlap with the area occupied with strong DA D2 blockers associated with EPS liability, and has limited overlap with areas covering sedative compounds, among this set of compounds which must all be regarded as monoamine modulators. Thus, based on this, it should be possible to find compounds with the desirable combination of optimal antipsychotic efficacy and minimal EPS liability and sedative properties, by designing compounds with an overall in vivo profile suggesting similarities with antipsychotic compounds, including some degree of DA D2 antagonism as reflected by the neurochemical indices, but avoiding the areas in the main model associated with EPS liability or sedation, and avoiding the behavioral inhibitory regions.

Dopidines. The dopidines, as well as novel compounds including IRL790 and IRL626, were developed with such a profile in mind. In the main model (Figures 1 and 2), they are located adjacent to the main "antipsychotic" cluster, offset from the strong D2 antagonists, as well as from the more sedative compounds such as clozapine and quetiapine, reflecting moderate effects on dopamine metabolites, combined with slight increases of serotonergic indices and a tendency to subtle behavioral stimulation. Due to the latter, along with some shared effects on cortical amines, the dopidines also cluster close to cognitive enhancers. The behavioral effects provide a clear distinction vs conventional dopamine D2 antagonists, as well as most of the atypical antipsychotics, which are markedly inhibitory. One notable exception to the latter are the benzamides, in particular amisulpride, which do resemble the dopidines in the models shown here, although the dopidines consistently display more pronounced neurochemical effects.

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Based on mapping as presented here, antipsychotic, and potentially procognitive properties, without adverse motor effects, were anticipated for the dopidines, as subsequently corroborated by extensive preclinical and clinical studies.[22−25,53−59]

In Vivo Systems Response Profiling. In general, this type of systems response map provides a basis for quantitative, systematic translational mapping across species and models. With traditional preclinical in vivo models, including specific behavioral assays, a predefined readout is typically postulated to correspond to a certain therapeutic effect, an approach that to a large extent has resulted in disappointment and hence the notion that in vivo models are unreliable and not predictive.[60] The approach presented here utilizes the full spectrum of readouts available from the in vivo experiments, rather than single end-points, and the array of phenotypic descriptors obtained can be fed into proper quantitative activity−activity relationship (QAAR) models, governed by functional data rather than by mechanistic hypotheses. In terms of conventional drug discovery workflow, it integrates the steps of lead discovery, lead optimization, and candidate selection, and may also cover one or both of the preceding steps, target identification and validation, although it is equally applicable in drug discovery projects with predefined, molecular targets. The PLS models can be regarded as a means of data compression, yielding readouts that can be directly correlated to, e.g., data on clinical properties, or to in vitro data, or molecular descriptors for QSAR work. The present study is restricted to neurochemical and behavioral data from acute experiments, but can be readily combined with, e.g., data collected from disease models, transgenic animals, chronic treatment setups, etc. This could be a way to address the problem of genetic heterogeneity, and heterogeneity regarding underlying pathophysiological mechanisms, which is likely present for many CNS disorders, such as depression and schizophrenia, and hampers the applicability of experimental disease models. The neurochemical analytes, albeit closely linked to monoamine neurotransmission, are not primarily viewed as direct measures of, e.g., transmitter release or specific synaptic events, but display response patterns that discriminate different states and drug effects across compound classes. For instance, specific response patterns of dopamine and its metabolites are well established for dopamine antagonists and agonists.[61] Another example is the characteristic pattern of changes in monoaminergic indices induced by the NMDA antagonist MK-801.[62]

The general principle of considering arrays of biomarkers rather than single end-points from in vivo assays, to improve differentiation and understanding of effects of antipsychotics and psychostimulants, are exemplified in a recent report on transcriptome fingerprints of intermediate early genes.[63] Another study applied proteomic analysis upon chronic administration to compare the system level effects of the antipsychotics clozapine, as compared to risperidone, suggesting, i.e., unique effects of clozapine on proteins involved in calcium homeostasis.[64] Such proteomic and transcriptomic response profiles could be integrated with neurochemical and behavioral data to help elucidating action mechanisms of CNS drugs, and improve predictivity in the drug discovery process.

It should be noted that the multivariate analysis approach applied, i.e., PLS regression on dose response data, is suited to pick up the main direction of the dose dependent effects. Thus, if biphasic effects are present, selection of a dose range with monophasic effects improves the sensitivity. Furthermore, the outcome is governed by the test compounds included, and thus, it is a relational map rather than a "GPS", which gives the possibility of very specific comparisons, but with the potential disadvantage that the coordinates of a specific compound change depending on which other compounds are analyzed in the same model. If a static map is desired, alternative analytical approaches can be applied, e.g., based on principal component analysis of data on a set of reference compounds selected to span the map.[65] New compounds can then be introduced in the same model, without affecting the original weights. As a general caveat, with respect to translational modeling, while it is appealing to be able to capture complex net effects in a relevant, physiological system, issues such as the presence or absence of species specific pharmacologically active metabolites, or otherwise differential PK properties, should be kept in mind. Limited or delayed CNS exposure can influence the outcome, even though the setup is tailored to pick up directions as well as amplitudes of dose dependent effects. In this case, all compounds tested do show robust CNS effects, in terms of either neurochemical or behavioral changes. An interesting extension of the present work could be to create models linking exposures and responses; however the compounds assessed herein have complex, and not fully understood action mechanisms, which limits the applicability of highly mechanistic PK/PD models. On the other hand, extending with exposure data would help elucidating different mechanisms.

■ CONCLUSIONS

We describe the application of systematic in vivo systems response profiling/phenotypic screening in the context of drug discovery as well as general pharmacological studies. Use of in vivo assays reduces the risk of missing relevant effects occurring due to e.g. downstream effects and interactions relating to the primary molecular target(s). Collection of large arrays of biological descriptors, rather than single end-points, improves sensitivity and resolution. The systematic, standardized workflow enables comparisons and classification, and creates a framework for translational mapping as well as QSAR modeling. The approach is exemplified by the analysis of dopidines, a new class of dopamine modulating compounds, compared to other classes of monoamine modulating compounds including antipsychotics, antidepressants, and psychostimulants, showing that the dopidines display a distinct phenotypic profile, suggestive of antipsychotic and possibly pro-cognitive effects, without motor inhibition in the normal state. This attractive and useful profile for treatment of several CNS disorders would not be possible to discover by traditional, mainly in vitro, target based screening.

Of note, this could be readily deduced based on data from intact rats, i.e., no specific disease models were used. The profile has prompted the investigation of dopidines in various more specific behavioral models, as well as in clinical studies.[22−25,53,55,59,66] Other phenotypically distinct classes indicated by the present analysis include cortical enhancers (e.g., IRL752) and a group of behaviorally stabilizing dopamine D2 receptor modulators not classified as dopidines (e.g., IRL790). In this study, standard, linear chemometric methods, PLS and PCA, are applied, focusing on multivariate dose response analysis; however, alternative techniques such as hierarchical analyses and machine-learning methods could be used, as well as more complex models linking in vitro (in vivo) and clinical data.[67] One important advantage of the linear chemometrics methods used here is their abilitity to provide

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interpretations and understanding of the reasons for the patterns and clustering obtained in the models in terms of the underlying variables. Furthermore, the profiling and, consequently, the translational mapping and predictions of clinical properties of novel compounds could be improved by extending the array of biological markers and collecting response profiles also in disease states, as well as incorporating exposure data.

METHODS

In Vivo Dose Response Analysis. *Animals.* Male Sprague–Dawley rats from B&K Scanbur (Sollentuna, Sweden), Charles River (Köln, Germany), or Taconic (Ejby, Denmark) were used. Rats weighed 160–180 g at the time of arrival. Rats weighed 220–260 g at the time of the locomotor and tissue neurochemistry studies. Animals were housed five animals per cage with lights on between 06:00 and 18:00, at 22 °C, with free access to food and water. All experiments were carried out in accordance with Swedish animal protection legislation and with the approval of the local Animal Ethics Committee in Gothenburg.

Drugs. The animals in the experiments were allocated into one of five treatment groups, $n = 4$, according to a latin square design to reduce the risk of effects of home cage. The treatment groups consisted of Vehicle (0.9% w/v NaCl or Glucose 5.5% w/v) and the compound tested at four doses, except in five cases were three doses where given, using five animals per group (see Supporting Information Table S3). Compounds were dissolved in physiological saline (0.9% w/v NaCl) or a few drops of concentrated HAc and 5.5% glucose and injected subcutaneously in a volume of 5 mL/kg 4 min before start of locomotor activity recording. Test compounds used in the analyses presented herein were as follows: lurasidone, cocaine, ephedrine, D-amphetamine, MDMA, desipramine, DOV21947, maprotiline, fluoxetine, venlafaxine, bupropion, citalopram, clomipramine, fluvoxamine, imipramine, mianserine, mirtazapine, reboxetine, sertraline, zimelidine, amitriptyline, tianeptine, aripiprazole, fluphenazine, iloperidone, n-desmetylclozapine, ziprasidone, bifeprunox, clozapine, quetiapine, remoxipride, risperidone, amisulpride, chlorpromazine, olanzapine, sulpiride, LY354740 (eglumegad), haloperidol, JNJ-37822681, raclopride, methylphenidate, atomoxetine, guanfacine, tacrine, galantamine, memantine, donepezil, (−)-OSU6162, pridopidine, ordopidine, seridopidine, IRL547, IRL667, IRL678, IRL696, IRL744, IRL626, IRL752, IRL790, A77636, SCH23390, SDZ219-958 (SDZ PSD 958[68]), dihydrexidine, pergolide, biperiden, apomorphine, and pramipexole. (−)-OSU6162, pridopidine, ordopidine, seridopidine, and all IRL-compounds were synthesized in-house, and the other test compounds where obtained from commercial suppliers. IRL compounds were at least 98% pure as determined by LC-MS. Purchased compounds were of ≥98% purity as certified by the supplier. Synthesis methods for new IRL compounds are described in refs 32 (example 36, IRL667), 33 (example 1, IRL696), 34 (example 1, IRL 744, example 5, IRL752), and 31 (example 1, IRL790). The dose range was carefully selected, based on published data and prior experience on in-house compound series, to be pharmacologically relevant, i.e., to capture typical behavioral and neurochemical effects for each compound class. Doses are provided in the Supporting Information in Table S3. For the compounds studied herein, the effects persist for more than 1 h and t_{max} in terms of efficacy occurs within the 60 min postdose assessment period.

Locomotor Activity. Locomotor activity was recorded for 60 min in 55 × 55 cm^2 sound and light attenuating motility meter boxes, with a maneuvering space of 41 × 41 cm^2 (Digiscan activity monitor RZYCCM (16) TAO, Omnitech Electronics), generating a time series of x, y (horizontal activity) and z (vertical activity) coordinates sampled at 25 Hz. This time series was subsequently converted into a locomotor pattern by calculating 11 main variables based on the time series. Each main variable was calculated at seven sampling frequencies from 25 to 0.25 Hz and pooled into 15 min periods, generating a locomotor pattern matrix of each animal consisting of 308 variables.

Variables. Ve: Vertical activity, number of time points where the z-coordinate does not equal zero. Di: Distance traveled. Me: Meander, sum of all angle differences between adjacent position vectors (without sign). Mem: Meander divided by distance. Vem: Vertical activity divided by distance. Mo: Activity fraction, time in motion divided by time (hence a value between one and zero). St: Stops/starts, number of the times the velocity changes from zero to non-zero. Note that a stop is defined in such a way that (in the highest sampling frequency) a stop of 1/25 s counts as one stop and a stop of 5 s counts as one stop as well. Stm: Stops in the middle zone, i.e., more than 5 cm from the wall of the recording box. Mi: Fraction of time spent in "middle zone", more than 5 cm from the wall of the box. A value between one and zero. Moving rats never display zero values, and low values are displayed by rats resting in the corner. Vel: Average velocity, gives the same information as the distance variable. Acc: Average acceleration without sign.

The use of several sampling frequencies were based on the observation that this captures information related to qualitative behavioral features (unpublished data). This observation was made in the process of setting up the behavioral analyses, when data were analyzed in order to select the optimal sampling frequency. For example, it was noted that rats treated with two different psychotomimetic compounds, either MK-801 or D-amphetamine, yielding a similar overall degree of locomotor stimulation, could still be distinguished based on the distance traveled variable only, if several sampling frequencies were used. This strongly indicated that it was useful to keep the variables calculated at several sampling frequencies, to maximize the information captured from the behavioral recordings.

In the subsequent multicompound analyses, the following variables were excluded due to redundancy: Mem, Vel, and Vem calculated at the reduced sampling frequencies.

Postmortem Neurochemical Analysis. Immediately after the behavioral activity recording sessions, animals were decapitated and brains were dissected into striatum, cortex, and limbic region (containing the nucleus accumbens, both core and shell, most parts of the olfactory tubercle and ventral pallidum). Tissue samples were immediately frozen and stored at −80 °C until they were homogenized with perchloric acid (PCA) (0.1M), ethylenediaminetetraacetic acid (EDTA) (5.37 mM), glutathione (GSH) (0.65 mM), and α-methyldopamine (0.25 μM) as internal standard. A digital sonifier (Branson Digital Sonifier 250-D) was used to homogenize tissue from the striatum and limbic region. Cortex tissue was homogenized using an Ultra Turrax T25 homogenizer. All samples were centrifuged at 10 000 rpm for 10 min at +4 °C. Cortex tissue was filtered in Munktell filter paper 5.5 cm quality 1F. Tissue eluates were analyzed with respect to tissue concentrations (ng/g tissue) of the monoamine transmitter substances (norepinephrine (NA), dopamine (DA), 5-hydroxytryptamine (5-HT)) as well as their amine metabolites (normetanephrine (NM), 3-methoxytyramine (3-MT)) and acid metabolites (3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA)) by HPLC separations and electrochemical detection (HPLC/EC). Stock standards (DA, NA, 5-HT, 3-MT, DOPAC, HVA, HIAA, 500 μg/mL) and internal standard (AMDA 500 μg/mL) are prepared once every 3 months. 5-HT and 5HIAA are dissolved in Milli-Q water. DA, NA, DOPAC, NM, 3-MT, and HVA are dissolved in 0.01 M HCl. 5-HT, 5-HIAA, NM, and HVA are kept in a fridge; DA, DOPAC, NA, and 3-MT are kept in a freezer. Standard solution for analyses containing standards diluted in homogenizing solution to a concentration of 0.05 μg/mL is prepared daily. The HPLC/EC method is based on two chromatographic separations dedicated for amines or acids. Two chromatographic systems share a common auto injector with a 10-port valve and two sample loops for simultaneous injection on the two systems. Both systems are equipped with a reverse phase column (Luna C18(2), dp 3 μm, 50 × 2 mm i.d., Phenomenex), and electrochemical detection is accomplished at two potentials on glassy carbon electrodes (MF-1000, Bioanalytical Systems, Inc.). Via a T-connection, the column effluent is passed to the detection cell or to waste. This is accomplished by two solenoid valves, which block either

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the waste or the detector outlet. By not letting the chromatographic front reach the detector, better detection conditions are achieved.

The aqueous mobile phase (0.4 mL/min) for the acid system contains citric acid monohydrate 14 mM, sodium citrate 10 mM, MeOH 15% (w/w), and EDTA 0.1 mM. Detection potentials relative to Ag/AgCl reference are 0.45 and 0.60 V. The aqueous ion pairing mobile phase (0.5 mL/min) for the amine system contains citric acid 5 mM, sodium citrate 10 mM, MeOH 9% (w/w), MeCN 10.5% (w/w), decane-1-sulfonic acid 0.45 mM, and EDTA 0.1 mM. Detection potentials relative to Ag/AgCl reference are 0.45 and 0.65 V.

Data Compilation and Quality Control. Data quality is monitored in a semiautomated system. First, an automatic software filtering is applied to check for data consistency in format and magnitude. Second, the 308 locomotor variables are automatically calculated from the 25 Hz data generating LMA patterns for each individual. MVA monitoring of quality is performed by manually evaluating the control animals in each new experiment in relation to historical controls (n > 5000) in a number of automatically generated multivariate models, created by principal component analysis (PCA) on the behavioral variables, subject to zero mean/unit variance scaling. Similar PCAs are also calculated based on the neurochemical variables. In addition, the data for each dose response experiment is subject to separate PCAs. Outlier animals are manually marked as "weak" or "severe" as well as the whole experiment ("good"/"no good"). This facilitates the analysis of impact of outliers on overall results in subsequent data analysis, when several dose response experiments are analyzed simultaneously. In some cases, a whole experiment can be considered to be of poor quality, e.g., due to aberrations in the control group. Data from such experiments are not used in the creation of multicompound data matrixes and subsequent analyses.

Data Analysis. The dose response data were analyzed by partial least-squares (PLS) regression,[16] applied to data matrixes organized with data from individual rats in rows, and variables denoting treatment and responses in columns. Treatment was represented by one variable for each compound, with the dose given as a dummy variable, i.e., 1, 2, 3, or 4 representing ascending doses. This means that a dependent variable block consisting of n variables was generated for an analysis of n different compounds. In one model covering 67 compounds, behavioral and neurochemical response data were combined, yielding an independent variable block of 248 variables. In a separate model of a smaller set of compounds created to specifically study compounds primarily affecting dopamine transmission, only behavioral response variables were included. Thus, the data matrix analyzed had 26 dependent and 228 independent variables. All independent variables were normalized to vehicle control group mean, and subject to zero mean and unit variance scaling, i.e., centered to zero mean and scaled to unit variance, and to log transform. In models combining neurochemical and behavioral data, block-scaling was applied, giving equal weight to the neurochemistry and behavioral variable blocks. For each compound, a dose response analysis using dose as dependent variable and the biological responses as independent variables was done, by PLS regression. Should a biphasic response be detected, further analysis can be improved by selecting a dose range in which the response is monophasic; however, for the dose response data presented herein, such adjustments were not needed. Compounds with a significant dose response relationship established by PLS were included in the subsequent, multicompound dose response analyses. PLS models with multiple Y variables equaling the number of test compounds included in data set were then generated. Statistical significance was established by cross-validation, leaving one-seventh of the rows/observations out in each round of cross validation.[16] Models were carefully checked with respect to potential impact of outliers, by examining residuals and object score plots, and recalculation with outliers excluded to further assess the stability of the results. All PLS modeling was performed using the Simca 13.0 software (Umetrics AB). In the results graphs presented below, neurochemical variables are denoted by abbreviated analyte (DO = DOPAC; HI = 5-HIAA; HV = HVA; MT = 3-MT; HT = 5-HT) followed by region (L = limbic region, S = striatum, C = cortex), i.e., DOL denotes DOPAC in the limbic area, etc.

Auxiliary Data. As an aid in the interpretation of results, published data on in vitro binding affinities and clinical efficacy and side effect liability were used.

Receptor Binding Affinities. The data set provided in ref 69, containing receptor binding affinity data on current antipsychotics data, was used as major source regarding in vitro binding profiles.

Clinical Effect Profiles. Effects sizes for antipsychotic effect and side effects were collected from ref 44, providing a comprehensive metaanalysis based on a total of 212 clinical studies including statistical estimates of overall efficacy, weight gain liability, sedation, prolactin increase, extrapyramidal side effects, and QTc prolongation for the following compounds assessed herein: haloperidol, amisulpride, clozapine, olanzapine, risperidone, aripiprazole, lurasidone, chlorpromazine, and ziprazidone.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.6b00371.

List of the doses used for each test compound and results from the multivariate analyses including complete $w*c$ weights for all components extracted (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Mailing address: Integrative Research Laboratories, A Wallgrens backe 20, SE-413 46 Gothenburg, Sweden. Phone: +46 730 757702. E-mail: susanna.waters@irlab.se.

ORCID

Susanna Waters: 0000-0002-8688-880X

Author Contributions

S.W., H.P., J.K., P.S., and N.W.: Wrote manuscript, designed research, and analyzed data. Y.S.: Wrote manuscript, analyzed data. T.A.: Wrote manuscript, designed research, performed research, and analyzed data. E.L.: Designed research, performed research, and analyzed data. C.S.: Wrote manuscript and designed research.

Notes

The authors declare the following competing financial interest(s): Susanna Waters, Peder Svensson, Johan Kullingsjö, Henrik Pontén, Theresa Andreasson, Ylva Sunesson, Elisabeth Ljung, Clas Sonesson, and Nicholas Waters are equity holders of Integrative Research Laboratories Sweden AB, which holds IP rights regarding IRL denominated compounds. Susanna Waters, Peder Svensson, Johan Kullingsjö, Elisabeth Ljung, Clas Sonesson, and Nicholas Waters are employed at Integrative Research Laboratories. Susanna Waters, Nicholas Waters, and Clas Sonesson are inventors of the patents covering dopidines. These patents are owned by Teva Pharmaceutical Industries, Ltd.

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